This protocol is the classic Hoffman Winston DNA preparation using bead bashing with amendments based on the Dunham Lab.

All spins are max speed in the microfuge.

Make a master mix of 400µL TE + 30µg RnaseA (from 100µg/µL stock in 4C room) based on the number of samples.

Be careful using phenol.

1. Grow an overnight culture in 5 ml YPD.
2. Spin 2 x 1.5mL to pellet in microfuge for 2 minutes. Decant supernatant. (Want to collect around 3 to 10 e8 cells)
3. Resuspend pellet in 500 µl water and transfer to a 2.0 mL tube. Or if desired you can store the cells in a Sorbitol solution and store at -80C.
4. Spin 2 minutes to pellet. Remove the supernatant, leaving just enough to resuspend the pellet completely.
5. Working in the fume hood, add to resuspended pellet:
   a. 200µl lysis buffer (recipe below)
   b. 200µl 25:24:1 phenol/chloroform/isoamyl alcohol (ensure that it is the buffered phenol).
6. Add mixture to 300mg acid-washed glass beads in a 1.5 ml Tube.
7. Vortex 8 minutes in the fumehood.
8. Touch spin in a low speed minifuge to get the phenol off the lid.
9. Add 200µl TE. Invert to mix.
10. Spin 5 min to separate aqueous and organic layers.
11. Transfer aqueous (top) layer to a new tube.
12. Add 1mL room temp 100% ethanol. Invert to mix.
13. Spin 2min. You should see a white pellet.
14. Remove supernatant and add 400µl TE+30 µg RnaseA.
15. Resuspend pellet using pipette tip and vortexing.
16. Incubate 30 minutes at 37C
17. Add 10µl 4M ammonium acetate and 1mL room temp 100% ethanol. Invert to mix.
18. Spin 2 min.
19. Remove supernatant completely and dry pellet by leaving the tube inverted on a kimwipe on the bench for 30 min.
20. Resuspend in 50µl Nuclease-Free H2O or TE.
21. Measure DNA concentration using a fluorometer.

Total yield should be 10-20µg.

**Lysis buffer**

- 2% Triton X-100
- 1% SDS
- 100 mM NaCl
- 10 mM Tris pH 8
- 1 mM EDTA

**Sorbitol Solution**

- 0.9 M Sorbitol
- 100 mM Tris pH 8
- 100 mM EDTA